

Susceptibility of Waxy Starch Granules to Mechanical Damage

A. D. Bettge,^{1,3} M. J. Giroux,² and C. F. Morris¹

ABSTRACT

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Starch samples isolated from wheat flour that represented four possible waxy states (0, 1, 2, and 3-gene waxy) were subjected to crushing loads under both dry and wet conditions. Calibrated loads of 0.5–20 kg were applied to the starch samples and the percentage of damaged granules was visually determined. Under dry crushing conditions, starches containing amylose (0, 1, and 2-gene waxy) had between 1% (5-kg load) to 3% (15- and 20-kg load) damaged granules, whereas waxy starch (3-gene waxy; <1% amylose) began rupturing at 0.5-kg load (3.5% damaged granules) and had 13% damaged granules when ≥10-kg load was applied. Under wet crushing conditions, normal and partial waxy starch (0, 1, and

2-gene waxy) showed little difference in percentage of damaged granules when compared to the results of dry crushing. Waxy starch (3-gene waxy), however, showed substantially increased numbers of damaged granules: 12% damaged granules at 0.5-kg load, rising to 55% damaged granules at 15-kg load. The results indicate that waxy starch granules are less resistant to mechanical damage than normal starch granules. Furthermore, blends of normal and waxy wheats or wheat flours intended to have a particular amylose-amylopectin ratio will be a complex system with unique processing and formulation considerations and opportunities.

Starch is the primary component of wheat flour (*Triticum aestivum*, L.) and plays an important role in many temperature-dependent interactions with water, including gelatinization, pasting, and retrogradation (Atwell et al 1988). Starch is comprised of amylopectin and variable amounts of amylose (Zeng et al 1997). The amylose component is synthesized by granule-bound starch synthase I (GBSS, EC 24.1.21). Due to the allohexaploid nature of wheat, various combinations of the three homoeologous GBSS or waxy genes may occur. When all three GBSS genes are present and functional, the grain is referred to as having normal starch. When only one or two GBSS genes are functional, the grains have intermediate levels of amylose and are referred to as partially waxy. Generally, 0-gene waxy (normal) wheat has ≈22–23% apparent amylose; 1-gene waxy wheat has ≈19–20% apparent amylose; 2-gene waxy wheat has ≈18% apparent amylose. When all three genes are absent (3-gene waxy wheat), the starch is essentially comprised of <1% apparent amylose (>99% amylopectin), and the grain is referred to as being completely or fully waxy. In this manner, amylose content roughly corresponds to GBSS gene dosage (Nakamura et al 1995, Zeng et al 1997). Many functional aspects of starch and flour, and many end-use qualities are affected by the amylose content of starch. Texture and quality of white salted (udon) noodles is better in partial waxy wheat as compared with normal types (Wang and Seib 1996, Batey et al 1997, Briney et al 1997). In bread, the rate of starch retrogradation and staling may be manipulated by adjusting the amylose content relative to amylopectin (Schoch 1965). Bread with higher amylopectin content may be more prone to staling and thus should be avoided. Extruded, expanded snack foods are also dependent on the content of amylose and amylopectin. Amylose forms films and produces a stronger, crunchy texture. Amylopectin produces products with greater volume and crispiness (Wang 1997).

Although waxy-type grains have been long known to exist in other crops such as maize (*Zea mays*), it has only been in recent times that fully waxy wheat has become available (Nakamura et al 1995). The availability of wheat starch (and flour) comprised of essentially 100% amylopectin presents challenges and opportunities

to the milling and food industries. Differences in rheological properties among normal, partial waxy and fully waxy wheat and other cereal starches suggest that granule structure and molecular organization differ significantly. Pasting as measured by the Rapid Visco Analyser (RVA) or viscoamylograph indicates that partial waxy samples gelatinize at temperatures similar to those of normal starch, but that fully waxy granules are less stable and gelatinize at lower temperatures (Hayakawa et al 1997, Morris et al 1998). Partial waxy starches tend to create higher viscosity pastes (Tester and Morrison 1992, Hayakawa et al 1997, Zeng et al 1997, Morris et al 1998), indicating that the amylopectin molecules interact with more water. Greater starch swelling, when heated in excess water, is also associated with reduced amylose (Crosbie 1991, Morris et al 1997), as is the manner in which lipids associate with the starch molecules (Morrison et al 1993).

In theory, the final amylose content of any product or process could be manipulated between 0% (using waxy starch) and ≈25% (using normal starch) on a starch basis. However, such binary mixtures may not be expected to necessarily perform like partial waxy starches, where all granules share more or less the same amylose content. To better predict how mixtures of starch types will perform, the physical and chemical characteristics of fully waxy wheat starch need to be defined. The physicochemical, structural integrity of waxy starch granules is one aspect that will affect utilization. The structural and organizational differences among cereal starches that differ in amylose content suggested by the pasting and rheological studies also may impart differences in resistance to mechanical stress. Fractured and cracked granules contribute to greater water absorption and therefore affect dough handling and rheology. In bread, a modest level of starch damage is beneficial because a greater amount of water may be added to a dough while maintaining adequate handling properties. Furthermore, damaged granules are also more susceptible to attack and hydrolysis by α -amylase when compared with intact granules; some starch hydrolysis is desirable to provide substrate for yeast respiration in panary fermentation. However, too much damaged starch leads to sticky doughs that are difficult to handle and have reduced water-holding ability (Gibson et al 1992). Crumb structure deteriorates, and the sticky texture interferes with slicing. In noodles, increased starch damage leads to undesirably firmer and duller colored noodles (Oh et al 1985, Elbers et al 1997). Dombrink-Kurtzman and Knutson (1997) hinted that differences in susceptibility of maize starch granules to physical damage might be due to differences in amylose and amylopectin contents. Reduced amylose content in maize starch granules was associated with greater susceptibility to physical damage by an electron microscope's beam; the resistance of granules to wrinkling and fracturing with exposure to the electron beam appeared to decrease with decreasing amylose. This

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² Department of Plant Sciences, Montana State University, Bozeman, MT 59717-3150.

³ Corresponding author. Phone: 509-335-4062. Fax: 509-335-8573. E-mail: abettge@wsu.edu

study examined the resistance of full and partially waxy and normal wheat starch to mechanical stress (crushing) in wet and dry environments (with and without water).

MATERIALS AND METHODS

Waxy wheat was produced by crossing Bai Huo (null at *Wx-D1*) and Kanto 107 (null at *Wx-A1* and *Wx-B1*) (Nakamura et al 1995; Morris and Konzak 2000, 2001). The presence of waxy starch was confirmed by iodine staining (0.1M I₂ with 0.1M KI) (AOAC 1990). Madsen (normal type; three functional GBSS enzymes), Penawawa (1-gene *waxy*; null at *Wx-B1*), Kanto 107 (2-gene *waxy*; null at *Wx-A1* and *Wx-B1*) were used in addition to the full waxy starches (3-gene *waxy*; null at all three loci). Two samples of each type, from two different millings, were used for prime starch production and two replicates of each sample were made for each crushing (*n* = 4 for each crushing load; wet and dry). All samples were soft wheat types. Wheat was milled with a modified Brabender Quadrumat system to produce straight-grade flour (Jeffers and Rubenthaler 1979). Prime starch was aqueously extracted from the

flour (Wolf 1964) and, after drying at ambient temperatures, sieved through a 150-μm screen.

Starch granule mechanical strength was compared by subjecting starch granules to compression. Starch granules (2.5 μL, 10 mg of

TABLE I
Damaged Starch Granules (%) Resulting from Dry Crushing^a

Compressive Load (kg)	Functional GBSS Enzymes ^b			
	0	1	2	3
0	0 (0)	0 (0)	0 (0)	0 (0)
0.5	4 (1.3)	0 (0)	0 (0)	0 (0)
1	4 (2.6)	1 (0.9)	0 (0)	0 (0)
2	9 (2.4)	2 (1.3)	0 (0.5)	0 (0.5)
5	11 (2.1)	2.5 (0.6)	1 (0.6)	1 (0.8)
10	13 (5.0)	3 (3.1)	2 (2.1)	1 (1.5)
15	10 (2.9)	1 (1.9)	1 (0.6)	2 (1.3)
20	10 (3.6)	1 (1.2)	1 (1.0)	1 (1.4)

^a Standard deviation in parentheses.
^b Granule-bound starch synthase I (GBSS, EC 24.1.21).

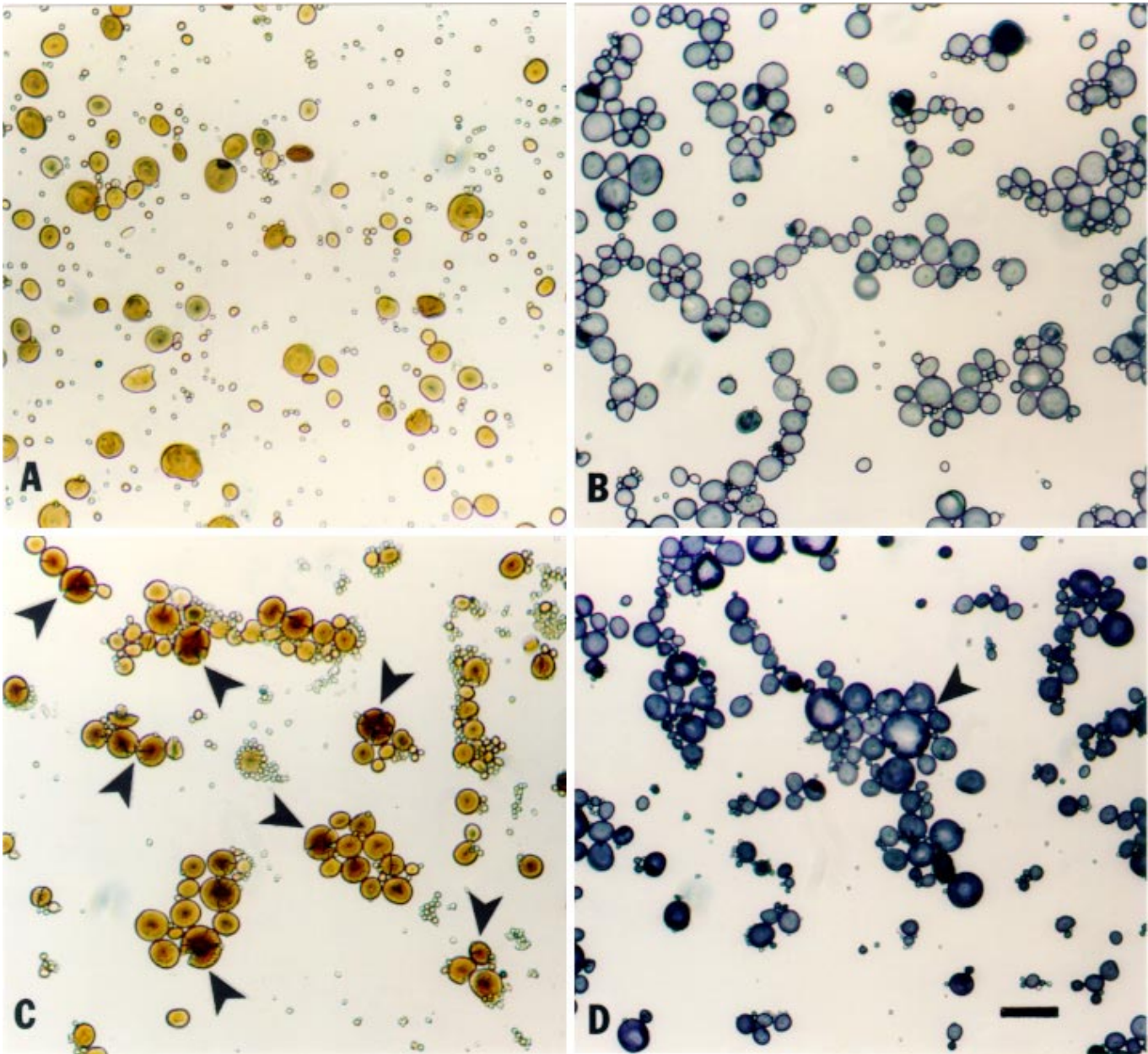


Fig. 1. Photographs of prime starch from waxy and normal wheat stained with iodine: A, waxy starch uncruushed; B, normal starch uncruushed; C, waxy starch after application of a 20-kg load; D, normal starch after application of a 20-kg load. Arrows indicate some damaged granules. Bar = 25 μm.

TABLE II
Damaged Starch Granules (%) Resulting from Wet Crushing^a

Compressive Load (kg)	Functional GBSS Enzymes ^b			
	0	1	2	3
0	0 (0)	0 (0)	0 (0)	0 (0)
0.5	12 (1.5)	3 (1.0)	1 (1.2)	1 (0.6)
1	14 (1.7)	2 (2.1)	1 (1.0)	2 (1.0)
2	15 (3.3)	2 (1.5)	1 (1.0)	0 (1.0)
5	22 (4.9)	4 (2.4)	2 (1.4)	2 (0.8)
10	34 (7.3)	4 (1.5)	4 (1.0)	3 (1.3)
15	55 (20.0)	4 (2.1)	2 (3.3)	1 (1.0)
20	51 (11.6)	2 (1.0)	2 (1.3)	2 (1.3)

^a Standard deviation in parentheses.

^b Granule-bound starch synthase I (GBSS, EC 24.1.21).

starch in 1 mL of H₂O suspension) were applied to glass microscope slides, spread to ≈0.5 cm diameter, allowed to air-dry, covered with a glass slip, and then crushed dry, followed by staining with 2.5 μL of iodine stain before microscopic examination. Alternatively, the samples were allowed to dry, as above, then treated with 2.5 μL of iodine stain, covered with a glass slip, crushed wet, and then examined under magnification. Compressive load was applied with a texture analyzer (TA.XT2 Texture Technologies Corp., Scarsdale, NY) at 0.5, 1, 2, 5, 10, 15, and 20 kg using a 1-cm² square probe moving at 0.1 mm/min. Photographs were taken of the stained granules at 200× magnification, and the percentage of broken granules was visually determined. Statistical modeling, analysis of variance (ANOVA), and mean separation analyses were made using PC-SAS 6.12 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Previous tests made on waxy wheat have suggested that waxy starch granules might be substantially more susceptible to physical damage than granules containing amylose (as in partially waxy or normal wheat). Reduced time to RVA pasting peak, greater breakdown, and lower final viscosity, in addition to reduced cookie spread (data not shown), all indicated that the physical granule structure of waxy starch may have less structural integrity under stress than does normal starch, in addition to having different granule starch composition (Zeng et al 1997).

Applying a calibrated load to isolated starch granules confirmed that waxy starch granules are indeed less resistant to mechanical crushing (Fig. 1). When crushed dry, normal starch granules began to rupture at 5-kg load (Table I). The percentage of granules ruptured rose from ≈1% at 5-kg load to <3% at 15- and 20-kg loads. Waxy granules, however, began rupturing at 0.5-kg load with 3.5% damaged granules. As the load increased, more waxy granules ruptured: ≤13% at 10-kg loads, with the largest increase occurring between 0.5- and 5-kg load. Because the granules were crushed dry, similar effects might be observed during grinding or milling. Because wheat kernels are crushed between rolls during flour milling, it is likely that waxy wheats will produce flours with increased physical damage to starch granules. Because waxy starch has no amylose, long regions of interpolymers hydrogen bonding and double helix formation, as is seen in amylose in normal wheat starch within the amorphous region of the starch granule, does not occur. Reduced hydrogen bonding may lead to the decreased resistance to crushing and concomitant increase in starch damage. As a result of aqueous starch extraction and fractionation (Wolf 1964), waxy prime starch shows no damaged granules before crushing, as much of the damaged starch was removed in the tailings fraction. Starch damage no lower than ≈2% was observed for waxy wheat flour when the flour was stained and microscopically examined before aqueous starch expression (data not shown).

Adding water to the system during crushing significantly amplified the damage to waxy starch granules at any given crushing load (Table II). Water may enhance the disruption of the crystalline

structure that occurs between the short stretches of the linear glucose polymer in amylopectin, leading to decreased mechanical strength of waxy starch granules. Conversely, water seems to have little effect on normal starch. The difference in starch damage between crushing normal and partial waxy starches (0, 1, or 2-gene nulls), dry and with water, was negligible. Normal starch showed few to no ruptured granules at any crushing load, wet or dry. Waxy starch was immediately damaged by even a light 0.5-kg crushing load, 12% of waxy granules were ruptured, ≤55% granules were damaged at 15-kg load.

CONCLUSIONS

The susceptibility of waxy starch granules to rupture under mechanical load is a material property that will need to be considered when milling and processing waxy wheat. Any process that subjects waxy starch to crushing or shearing, particularly in an aqueous medium, will likely cause considerable starch damage and modification of end-use properties. These effects may be problematic or advantageous depending on how the processing is conducted. A particularly large effect may occur in extrusion processing, where higher amylopectin ingredients are desired for increased expansion coefficients and volume-forming properties (Wang 1997). If granule damage is not desirable, waxy starch may pose a problem. Modification of processing systems could lead to enhanced products if the intrinsic properties and process responses of waxy wheats are known and appropriately manipulated. Furthermore, the results suggested that using waxy wheat, flour, or starch in blends with normal wheat, flour, or starch to obtain reduced amylose content may have unexpected effects on end use. The waxy starch granules will be more susceptible to damage and paste earlier than will normal granules. A system composed of both waxy and normal starches would lead to a complex system with unique processing and formulation considerations and opportunities.

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